

Programmable Control of Nanoliter Droplet Arrays Using Membrane Displacement Traps

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A unique droplet microfluidic technology enabling programmable deterministic control over complex droplet operations is presented. The platform provides software control over user-defined combinations of droplet generation, capture, ejection, sorting, splitting, and merging sequences to enable the design of flexible assays employing nanoliter-scale fluid volumes. The system integrates a computer vision system with an array of membrane displacement traps capable of performing selected unit operations with automated feedback control. Sequences of individual droplet handling steps are defined through a robust Python-based scripting language. Bidirectional flow control within the microfluidic chips is provided using an H-bridge channel topology, allowing droplets to be transported to arbitrary trap locations within the array for increased operational flexibility. By enabling automated software control over all droplet operations, the system significantly expands the potential of droplet microfluidics for diverse biological and biochemical applications by combining the functionality of robotic liquid handling with the advantages of droplet-based fluid manipulation.

1. Introduction

Droplet-based microfluidic platforms offer powerful capabilities for discretizing and manipulating small aqueous sample volumes, with broad applications across the fields of chemistry,

materials, biology, and biomedicine.^[1,2] Droplet microfluidics technology has been particularly impactful in the development of novel biological and biomolecular assays, with the compartmentalization of individual sample volumes enabling new platforms for applications such as digital PCR,^[3,4] whole genome amplification,^[5–8] single-cell RNA sequencing,^[9–11] combinatorial biology,^[12] and high throughput screening.^[13–16] These assays typically rely on the generation of a set of monodisperse droplets in the nanoliter or sub-nanoliter range that serve as parallel reaction volumes, with monitoring of the chemical or biological reactions performed in a static chamber or in a continuous-flow channel via flow cytometry. Given their advantages over alternate methods of sample discretization, including low sample volumes, reliable droplet generation

with precise and uniform volume control, and integration with other microfluidic capabilities, there is significant interest in expanding the range of functional operations that can be performed within droplet microfluidic platforms. In particular, the ability to controllably position individual droplets within a microfluidic flow network, and manipulate their interactions with other droplets, would open the door to new capabilities for assay design and implementation.

Robotic liquid handlers are ubiquitous tools for laboratory automation due to their ability to provide precise software-defined control over multiple fluid extraction and dispensing steps, enabling flexible workflows that can be adapted to the needs of individual assays.^[17,18] Achieving this degree of control and customization for droplet microfluidics would enable multi-step assays with significantly reduced sample and reagent volumes compared with well plates, while simultaneously addressing other limitations of robotic liquid handling systems including system size and cost. One approach to programmable microfluidic control of discrete fluid volumes is the use of electrowetting-on-dielectric (EWOD) technology, in which droplet surface tension gradients are modulated using electrical biases to mobilize droplets over a planar substrate.^[19–22] Despite challenges with fabrication costs,^[23,24] reliability,^[25,26] and high voltage requirements,^[21] various EWOD platforms have been shown to provide highly flexible control over multiple droplet operations,^[21,27] and with the potential to

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operate using fluids with widely varying properties^[27] However, digital microfluidics using EWOD requires the use of large droplet volumes in the microliter^[19,25,28,29] to milliliter^[23,24] range for effective electrowetting-based actuation. To enable control over smaller droplet volumes, a variety of passive techniques based on nanoliter-scale water-in-oil droplet microfluidics can enable key sample manipulation steps such as droplet trapping and release.^[30] In these passive systems, droplets are selectively captured in discrete chambers using a combination of capillary and hydrodynamic forces,^[31–36] with droplet release commonly achieved by reversing the flow direction through the capture chambers. Droplet trapping^[37–40] and release^[41–43] have also been reported using various active methods including electrokinetic,^[44] optical,^[45] or pneumatic^[46,47] actuation, providing higher levels of droplet control than solely passive techniques. In addition to spatially constraining individual droplets, typical chemical or biochemical workflows also require the ability to combine multiple fluid volumes. Microfluidic droplet merging has been successfully demonstrated using passive elements such as constriction chambers,^[48] variable channel geometries,^[49–51] dual-chamber traps,^[52] and pillar structures.^[53] Active droplet merging has similarly been reported, with on-demand mixing between adjacent droplets performed using techniques including magnetic actuation,^[54] acoustic forces,^[55] electrocoalescence,^[56–58] and dielectrophoresis.^[59,60] In addition to merging, droplet splitting has also been explored by leveraging selective breakup of a larger droplet at a microfluidic junction.^[61–64] While these capabilities extend the toolbox for droplet microfluidics, the integration of multiple functions into a single device has proven challenging, since individual steps can require the use of different physical principles that are not readily combined in a single microfluidic system. The integration of passive droplet trapping, merging, and sorting steps has been described,^[65] but requires a continuous flow system where the sequence of operations is predefined by the static configuration of the microfluidic network. Recently, several promising systems employing pneumatic signals to directly control on-chip elastomer valves^[66] or modulate inlet flows^[67] have enabled additional flexibility in assay design. Despite these advances, droplet microfluidics as a platform for arbitrary manipulation of discretized nanoliter-scale sample volumes remains elusive.

Here we report a fully automated microfluidic platform offering flexible and deterministic control over the location and state of individual nanoliter-scale droplets, allowing complex sequences of discretization, metering, and mixing operations to be performed in a manner analogous to robotic stations employing automated pipetting for fluid control. The technology significantly extends the capabilities for automated droplet manipulation by enabling software-defined control over arbitrary sequences of complex droplet operations. The platform leverages membrane displacement trap (MDT) elements that allow direct modulation of trap volume to capture, split, meter, merge, mix, and eject selected droplets. An array of individually-addressable traps is combined with multiple sample inlets supporting dynamic on-demand droplet generation and an H-bridge valve topology enabling bidirectional flow control for arbitrary droplet positioning within the system. Using this simple and scalable chip design, the microfluidic devices are coupled with a vision system for automated droplet tracking and closed-loop control

of the on-chip valves. A scripting language provides control over user-defined droplet sequences, with optimized timing of the on-chip actuation elements enabling precise control over each process step without user intervention. The resulting platform opens new opportunities for deterministic control over multi-stage chemical and biological assays performed within nanoliter-scale droplets.

2. Results and Discussion

2.1. Chip Design and Operation

Each MDT trap consists of a 200 μm diameter circular well connected to a main channel by a short neck designed to prevent trapped droplets from becoming entrained in the main channel flow. An elastomer membrane beneath the well separates the trap from a hydraulic pressure control channel. When actuated, the membrane deforms into the trap and displaces fluid from the chamber, with the resulting volume change determined by the pressure applied through the hydraulic control channel. We previously demonstrated that this simple process can be employed to achieve reliable on-demand droplet capture and ejection from the MDT traps.^[47,68,69] In the present work we significantly extend the range of unit operations that can be implemented using MDT technology. An overview showing the configuration of a membrane displacement trap is presented in **Figure 1A**, and operation of a single trap for droplet capture and release is shown in **Figure 1B**. Extending this basic functionality, the full range of MDT unit operations investigated in this work is presented in **Figure 1C**. The overall MDT chip design includes an array of nine independent trap elements, two independent dynamic droplet generators, and a fluidic H-bridge circuit. A detailed view of a fabricated chip is shown in **Figure 2A**, and a schematic of the chip design together with the closed-loop control system including pressure sources, pneumatic actuators, vision system, and microcontroller is presented in **Figure 2B**. An image of the full experimental system including fluidic and pneumatic connections for droplet generation, manipulation, and H-bridge flow reversal is presented in **Figure 2C**. The digital camera and pneumatic valve actuator manifold shown in this image are connected to the controller code running on a desktop computer. The Python-based code, termed FluidScript, performs automated droplet position and volume measurements and controls all MDT actuator states in closed loop based on operational sequences specified in a user-defined script. When instantiated within a user script, the FluidScript code presents a user interface (**Figure 2D**) that includes raw video of the MDT trap array, interfaces for adjusting video settings and threshold values to allow optimization of droplet imaging for different solutions and lighting conditions, and a filtered video feed based on these settings. The filtered video panel also serves as an interface allowing the user to define arbitrary reference points and regions of interest that may be referenced in the script, for example to create trigger points for valve actuation. An overview of the code structure is presented in **Figure 2E**, and a summary of the class methods accessible to the user for script generation are available through Note S1 (Supporting Information). Droplet centroid position, length, and width (**Figure 2F**) are monitored and may be accessed within FluidScript, allowing

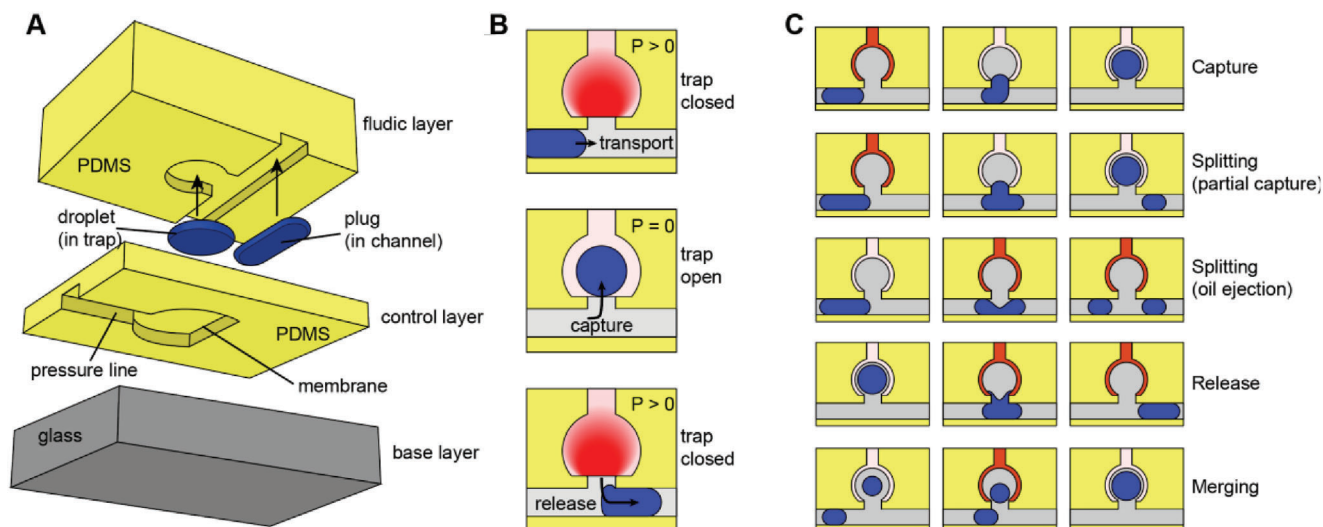


Figure 1. Membrane displacement trap arrays. A) Schematic view of a single trap element with an exploded view of the multilayer MDT structure. B) Simplified view of the droplet capture and ejection process, with pressure states in the hydraulic membrane actuation channel shown for each step. C) MDT unit operations including droplet capture, splitting by partial capture, splitting by oil ejection, release, and merging. Membrane actuation state is indicated by red (on) or yellow (off) shading of the control channel. Continuous phase flow in each panel is from left to right.

accurate droplet volumes to be determined in the user-defined script.

Two important aspects of the platform are the inclusion of parallel droplet generators for on-demand droplet production, and the introduction of novel microfluidic H-bridge elements capable of rapidly switching flow direction across the MDT array. Each dynamic droplet generator employs a T-junction combined with a membrane valve for injection of discrete aqueous volumes into an oil phase flowing within the main channel. This design provides precise control over the injected aqueous volumes.^[47,69] The independent plug generators allow two different samples to be injected at desired time points. Additional generators may be readily integrated for applications requiring higher levels of sample multiplexing. The microfluidic H-bridge circuit enables on-demand switching of flow direction through the trap array. The fluidic circuit is shown schematically in Figure 3. The actuation state for both sets of valves is defined using an off-chip three-way pneumatic valve, allowing flow direction to be switched from a single control output.

2.2. Vision System and Droplet Tracking

Droplet tracking is performed via custom vision system code written in Python using the cross-platform OpenCV image processing library. The resulting system is capable of real-time tracking of multiple droplets at ≈ 50 frames per second with an image resolution of 640×480 pixels. The vision system's blob detection algorithm yields geometry data for the projection of a droplet onto the chip surface. To extract droplet volume from this information, the 3D shape of the droplet must be estimated. There are multiple possible morphologies for a droplet, depending on its size and location within the MDT device. Referring to the channel and trap geometry presented in Figure 2F, the flow network consists of a main channel of width w and height h , with $h < w$, and the planar

projection of a droplet possesses a major axis length of $2a$. In the case of a small spherical aqueous volume with radius a smaller than half the channel height ($0 < a \leq h/2$), the volume may be directly determined from its radius via Equation (1):

$$V = \frac{4}{3} \pi a^3 \quad (1)$$

As the radius grows beyond $h/2$, the droplet becomes constrained by the upper and lower channel surfaces, resulting in a disc with a semi-toroidal perimeter. Under the assumption that the oil lubrication layer between the water phase and channel surfaces prevents wetting of the channel walls, the droplet volume for this configuration is found by integration (see Note S2, Supporting Information) to yield the expression presented in Equation (2), which is valid over $h < 2a \leq w$ for a droplet in the main channel, and $h < 2a \leq 2R$ for a droplet volume captured within a trap:

$$V = \frac{\pi h}{24} [24a^2 + 6h(\pi - 4)a - h^2(3\pi - 10)] \quad (2)$$

For a droplet within the main channel, any further increase in volume ($a > w/2$) will lead to the formation of an oblong plug with ends possessing 2D curvature defined by interactions with the channel walls in both the width and height dimensions. As modeled by Musterd et al.^[70] the plug volume in this case may be approximated by Equation (3):

$$V = \left[hw - (4 - \pi) \left(\frac{2}{h} + \frac{2}{w} \right)^{-2} \right] \left(2a - \frac{w}{3} \right) \quad (3)$$

A summary of the valid regions for each expression is provided in Figure S1 (Supporting Information). For each droplet tracked by the vision system, the value of a is extracted from the image data and used to determine the droplet morphology, followed by

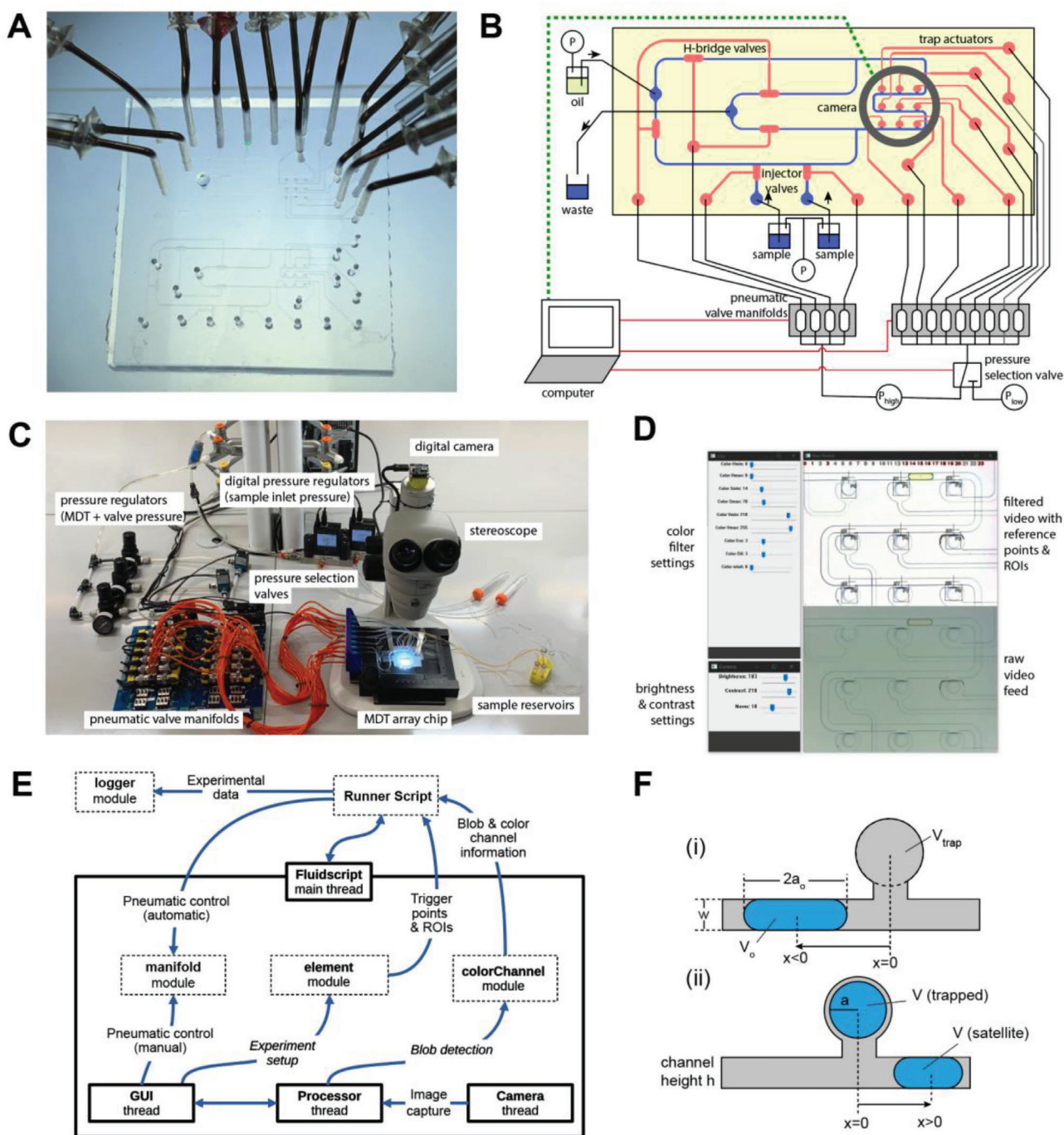


Figure 2. MDT device and system overview. A) Image of a fabricated chip containing two MDT arrays, with the upper device under test. B) Schematic of the closed-loop control system including the MDT array chip, vision system, and pneumatic actuators for on-chip valve operation. C) Image of the experimental system. D) Overview of the FluidScript graphical user interface. E) Schematic summary of the software operation. The runner script is user-defined Python code utilizing the custom FluidScript module to define all droplet operations. F) Geometric parameters used for droplet characterization i) before and ii) after a partial droplet capture event.

application of appropriate expression from Equations (1)–(3) to calculate the estimated droplet volume.

Time delays introduced by the software and hardware used for image capture, computation, and valve actuation can lead to error between the imaged droplet position and actual position at the

time of trap membrane actuation. The primary source of latency (λ) within the MDT system is mechanical compliance within the pneumatic system used to control the on-chip valves, including air compressibility, compliance within the tubing connecting the pressure source with the chip inlets, and compliance associated

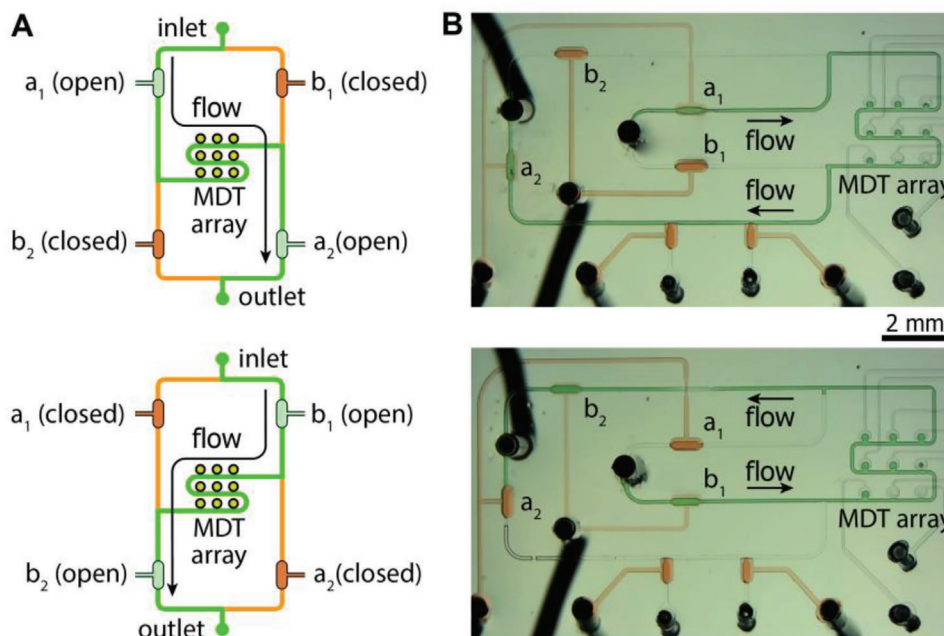


Figure 3. Microchannel H-bridge for bidirectional flow control. A) Schematic of the microfluidic H-bridge topology used to control the direction of fluid flow through the MDT array. The flow path is rapidly switched by inverting the opposed states of two pairs of on-chip elastomer valves, with valves a_1 and a_2 sharing one state (open or closed) and valves b_1 and b_2 sharing the opposite state. B) Images showing bidirectional flow through a fabricated chip, with valves labeled by the corresponding elements in panel A.

with the elastomeric chip itself, while secondary sources of latency include camera frame rate limitations and delays resulting from processing speed limitations for the OpenCV code. To account for error between the reported and actual droplet positions, the vision system code extracts droplet velocity (u) and observed position (x_{obs}) from the measurements, and estimates the true droplet position (x) as $x = x_{\text{obs}} + u\lambda$ at each measurement time point. Because velocity and position are continually monitored for all droplets within the user-defined detection regions, this approach ensures that accurate position estimates are generated regardless of flow velocity.

While latency remains constant for a given experimental configuration, changes in tubing connections or off-chip pneumatic valve apparatus result in variations in the observed time delay. Total latency for the given configuration was determined before implementing any experiments by generating a sequence of droplets with volume significantly smaller than the static trap volume, and capturing these droplets with a random distribution for x_{obs} . Because the distribution of capture events is expected to be symmetric about $x = 0$ due to the low flow rate within the main channel, latency can be directly quantified from asymmetry of the measured data. Using this approach, estimated latency for all experiments reported in this work ranged from $\lambda = 50$ –200 ms.

2.3. Droplet Generation

Software-defined droplet manipulation combines the flexibility of robotic microplate systems with microfluidics, enabling the execution of complex sequences of on-chip sample and reagent handling steps that can take advantage of the unique capabil-

ities of microfluidic technology. In the case of droplet generation, microfluidics can provide control over initial fluid volumes with picoliter precision, and enable unique droplet constructs such as multi-emulsion^[71] or Janus^[72,73] droplets. In the present work, initial droplets are generated by a previously-reported technique^[74–76] in which a membrane valve is used to gate a dispersed aqueous phase into a continuous-phase oil flow at a microfluidic T-junction,^[47,77–80] providing an effective method for precise software-defined control over droplet timing and volume. To establish the relationship between valve timing and droplet volume, experiments were performed at different continuous phase flow rates and gating valve opening times, with the resulting droplet volumes determined from the automated imaging code. An example of droplet generation with valve dwell time increasing from 50 to 600 ms is presented in Movie S1 (Supporting Information). In this movie, three droplets are generated for each valve dwell time to demonstrate repeatable volume control. Droplets ranging from 80 pL to 2 nL were reliably formed, with relative standard deviation below 5% over the full volume range (Figure 4A). The oblong valve body used by the droplet generator prevents leakage while the valve is in its off state owing to the high Laplace pressure imposed by the long and narrow side gaps along the edges of the valve body (Figure 4B), allowing reliable flow control without the need for complete sealing of the valve seat.

2.4. Droplet Capture

Following droplet generation, the membrane displacement traps enable multiple functions including droplet capture, ejection,

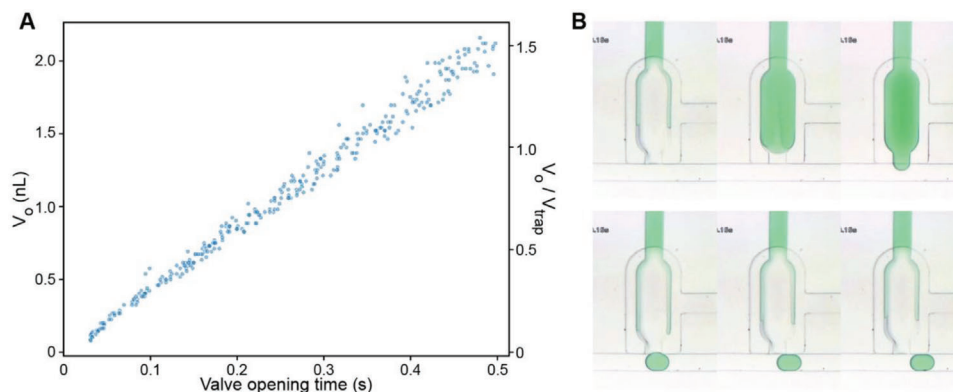


Figure 4. Droplet generation process. A) Discrete aqueous volumes generated by applying different valve opening times at the gated T-junction with a fixed oil flow rate of 141 nL min^{-1} . B) Sequential images of the droplet generation process for a 75 ms valve dwell time.

splitting, and merging. Software-controlled automation of these steps requires that appropriate valve timings for each action be determined. To investigate the impact of actuation timing on each unit operation, the automated system was programmed to implement each droplet manipulation step with random timing while tracking the resulting event. The first operation we explored was the capture of droplets with initial volume (V_o) smaller than the static trap chamber volume (V_{trap}). The latter term is defined by a cylinder with radius and height of the trap chamber, and does not include the volume of the entrance neck. In practice, the maximum volume that can be isolated within the trap without protruding into the main channel flow is less than the full trap volume, since deforming a droplet into the trap neck increases the interfacial free energy less than filling the internal corners of the trap chamber as the droplet volume expands. A sequence of droplets was generated with different half-length values (a_o) between 58 and $164 \mu\text{m}$, corresponding to volumes ranging from 0.35 to 1.25 nL . A single MDT trap was actuated with the droplet center located a distance x from the midline of the trap entrance, with x constrained to the range $|x/a_o| < 3$. To avoid experimental bias, values for trap opening position and droplet volume within the sequence were randomized. The resulting data is presented in Figure 4A. Full droplet capture is achieved for $|x/a_o| < 1$, with a steep loss in capture efficiency outside this range. While droplet capture is nearly binary, some splitting is observed at the transitions between capture states, with the split droplets clustered in regions with nearly equal volume between the parent and child droplets. We note that normalizing the position measurements by the static trap radius (a) was initially expected to serve as a suitable independent variable for the analysis of MDT actuator timing. However, it was found that normalizing by the half-length of the initial droplet (a_o) significantly reduced variability in the resulting relationships. As a result, this normalization is used for all position data in Figure 4.

2.5. Droplet Splitting

Droplet splitting can be performed by capturing an initial droplet with volume greater than the trap chamber volume (Figure 5B), yielding a child droplet with volume given approximately by

$V_o - V_{\text{trap}}$. In this case, the trap actuation timing is defined to select the desired portion of the droplet to be captured, with the secondary satellite droplet formed during splitting remaining in the main channel. The timing of the actuation step and initial droplet volume dictate the volume of the resulting primary and satellite droplets. The partial-capture process was studied by generating large droplets with $V_o > V_{\text{trap}}$, and attempting to trap these droplets at different positions while monitoring the parent and child droplet volumes generated by each splitting event. As with the previous experiments investigating the full capture of smaller droplets, the sequence of droplet volumes and actuation times was randomized. Initial droplets in this experiment possessed half-lengths between 181 and $365 \mu\text{m}$, corresponding to volumes ranging from 1.40 to 2.95 nL . The relationship between trapped volume and droplet position is presented in Figure 5B with captured volume normalized to the static trap volume. As seen in this figure, droplet capture events within the range of $|x/a_o| < 0.5$ tend to result in complete filling of the trap, ensuring a predictable volume for child droplets formed during the partial capture process. To confirm this expectation, droplets remaining in the main channel following each partial capture event when $|x/a_o| < 0.5$ were analyzed, as shown in Figure 5C. As expected, the resulting satellite droplet volume scales linearly with the initial droplet volume. The partial-capture droplet splitting technique is demonstrated in Movie S2 (Supporting Information). The resulting satellite droplets remain intact, with no secondary splitting events observed. A minimum satellite droplet volume of $\approx 0.2V_{\text{trap}}$ was achieved using this technique. Because the droplet splitting process is largely analogous to droplet generation in a T-junction channel geometry, the lower bound for satellite droplet volume is expected to depend on the hydrostatic pressures within the oil and water phases.^[76] As a result, the minimum satellite droplet volume is believed to largely depend on the channel and trap neck geometry, rather than the capillary number of the flow. Applications requiring smaller satellite droplets may thus benefit by further reducing the main channel height and minimizing the trap neck width.

As an alternative to partial capture for droplet splitting, a larger sample plug can also be split into two separate volumes by ejecting oil from an actuated trap as the plug traverses the trap opening (Figure 5C), destabilizing the droplet interface and

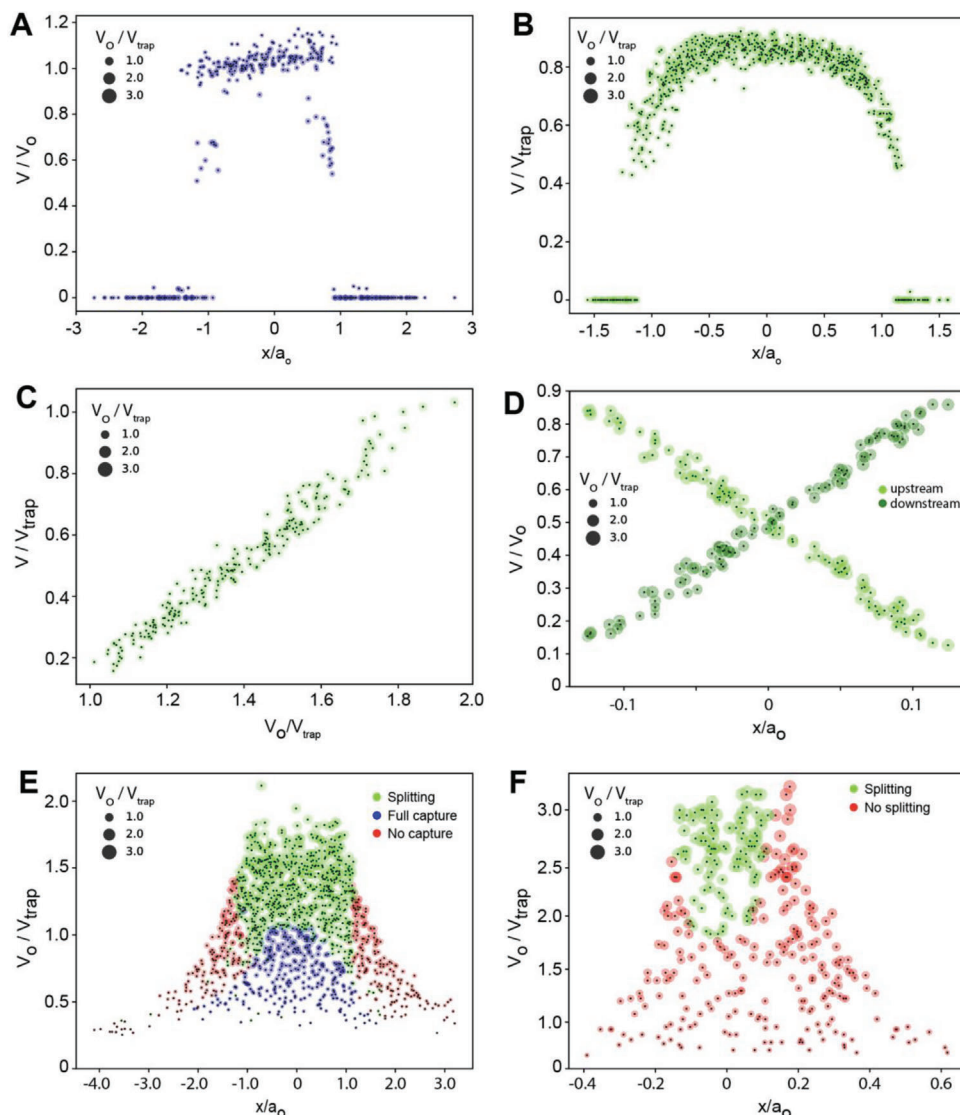


Figure 5. Droplet capture and splitting. A) Complete capture of small droplets with $V_o < V_{\text{trap}}$ ($n = 372$). B) Captured volumes from droplets with initial volumes larger than the static trap volume ($V_o > V_{\text{trap}}$), resulting in droplet splitting ($n = 650$). C) Satellite droplets resulting from the subset of partial capture events in panel B where the trap is filled above 80% of its static volume ($n = 225$). D) Droplet volumes generated via splitting of an initial sample plug by oil ejected from a trap ($n = 312$). Operational regimes defining the domains where splitting occurs are mapped for both E) partial capture and F) oil ejection, with clear boundaries between the different regions. Marker sizes are consistent across all plots, and represent the ratio of initial droplet volume before capture or splitting to the static trap volume (V_o/V_{trap}).

generating a pair of satellite droplets on either side of the trap neck. The volume of each child droplet is controlled by adjusting the timing of the oil ejection step. Droplet splitting data from a set of several hundred oil ejection events is presented in Figure 5D, and an example of this process is provided in Movie S3 (Supporting Information). Compared with the partial capture method of droplet splitting, oil ejection provides improved precision for the resulting satellite volumes with low variance. After splitting, the resulting satellite droplets are also readily recaptured in subsequent traps, as shown in Movie S4 (Supporting Information). However, the oil ejection approach was only effective for plugs with initial volume greater than nearly twice the trap volume, with smaller droplets displaced within the channel in-

stead of splitting regardless of position during oil ejection. This constraint is imposed by the trap neck width and main channel width, with reduced width values expected to enable the splitting of smaller droplets by oil ejection. Furthermore, droplet splitting could only be achieved over a narrow range of droplet position with the initial droplet centroid located within $\approx 15\%$ of its half-width from the trap midline. Actuating the MDT trap outside this window resulted in displacement of the intact droplet within the main channel without splitting.

Overall performance of the different splitting techniques can also be seen in the domain maps for partial capture and oil ejection presented in Figure 5E,F, respectively. These plots display the range of initial droplet volumes and trap actuation positions

that yield full droplet capture or droplet splitting for each unit operation. Significantly, the boundaries defining each domain are distinct and display minimal overlap. It is important to note that the domains are expected to be design-dependent, and changes to the channel or trap geometry will result in different operational regions. Thus, new MDT chip designs must be recharacterized to determine appropriate operation regimes and develop new analytic relationships needed to define actuation times for specific droplet manipulation steps.

2.6. Droplet Ejection

Once captured, droplets may be ejected from their traps by actuating the MDT membrane to reduce the trap volume and force the contents into the main channel. At the main channel flow rate used here, the change in trap volume is sufficiently rapid to expel the droplet without generating satellite droplets by flow-induced shearing. A typical capture and ejection sequence for a 0.44 nL droplet is depicted in Movie S5 (Supporting Information). When ejecting larger droplets approaching the maximum trap volume, rapidly switching the MDT control line pressure to its peak value occasionally resulted in droplet splitting, with a portion of the initial droplet remaining within the trap after completing the ejection step. This behavior occurs when the larger droplets are unable to fully exit the trap before the actuator membrane reaches its final position, resulting in pinching of the droplet tail from the ejected volume. To prevent unwanted droplet splitting, a two-stage ejection technique was employed in later experiments. In this process (Figure S2, Supporting Information), the pressure was first increased to 3.5 psi to partially expel the droplet from the trap while using the vision system to monitor the position of the droplet tail. When the tail neared the trap exit, the MDT control line pressure was increased to 20 psi. This two-step pressurization process reduces the effective capillary number during ejection to enable reliable droplet removal from the trap without splitting.

2.7. Droplet Merging

The controlled mixing of discrete fluid volumes is a fundamental step for chemical and biological assays. Passive droplet merging has been widely demonstrated in microfluidic flow systems by employing various geometries to bring droplets into interfacial contact either during flow^[81–88] or while confined in a static microwell,^[89–92] allowing them to merge and mix after draining the oil lubrication layer between the droplets.^[93,94] To achieve droplet mixing within the automated MDT arrays, multiple droplets are captured within a single trap, forcing the paired droplets into close proximity to promote interfacial contact and merging. Following an initial droplet trapping step, a second droplet is captured within the same well by partially actuating the membrane with an applied pressure of 2.5 psi before the target droplet reaches the trap, thereby reducing the trap volume without ejecting the initial droplet. As the second droplet reaches the trap entrance, the pressure is released, pulling the droplet into the trap to facilitate interfacial contact and ultimately droplet fusion after draining the oil layer between the discrete

volumes.^[93,94] The process of oil draining and droplet merging was observed to be nearly instantaneous for larger droplets with a combined volume approaching V_{trap} , while smaller droplets often required several seconds after coming into contact within the trap before merging. A sequence of images revealing the dynamics of the merging process is shown in Figure S3 (Supporting Information), and a sequence of repeated droplet merging events demonstrating the repeatability of the process is provided in Movie S6 (Supporting Information).

2.8. Flow Reversal

A unique aspect of the presented design is a bidirectional valve configuration that enables rapid switching of flow direction within the MDT array. This design is a fluidic analog of an electrical H-bridge circuit^[95] commonly employed for reversing the direction of current through DC motors or other electrical loads. Topologically, the flow circuit consists of two pairs of membrane valves positioned within a loop connecting the inlet and waste ports, and intersecting the MDT flow path on either side of the array (Figure 3). When actuated with a pair of inverted pressure inputs to hold one set of valves open and the other set closed, the two sets of valves serve to toggle between the opposing flow paths through the MDT array. The use of an off-chip three-way pneumatic valve to select the pressure applied to both sets of valves from a single control output provides a convenient approach for changing the flow direction. Compared to the use of multiple off-chip pumps and valves to change the flow direction, the H-bridge design significantly reduces system complexity, and was found to effectively minimize the fluidic dead volume and eliminate pressure perturbations during flow switching. Rapid reversal in flow direction was achieved (Movie S7, Supporting Information), with measurements of switching speed limited only by the camera frame rate. Demonstrations showing the utility of flow reversal for manipulating droplets within the MDT array can be seen in Movies S8 and S9 (Supporting Information), which present example sequences of automated droplet sorting steps. Images depicting the sequence of droplet states achieved in Movie S9 (Supporting Information) are presented in Figure 6.

2.9. Flow Rate Limitations

Maximum droplet processing speed in the MDT array is primarily constrained by the flow rate used to mobilize droplets in the main channel. All droplet handling steps reported in this work were performed at a bulk flow rate of 141 nL min^{−1}, corresponding to a flow velocity of 470 μm s^{−1}. One potential constraint limiting the flow rate is the capillary number (Ca) of the system, which scales with flow velocity. At higher capillary numbers, viscous forces can be sufficiently large relative to surface tension to impact droplet generation. Using an estimated interfacial tension value of 20 mN m^{−1} for our surfactant system,^[96] the resulting capillary number for flow in the main channel is calculated as $Ca = 2.1 \times 10^{-5}$, well within the range of $Ca \ll 0.01$ reported for stable droplet formation in microchannels with similar geometry as the present MDT devices,^[76,97] suggesting that significantly higher flow rates range may be employed without sacrificing droplet stability. A more practical parameter limiting flow

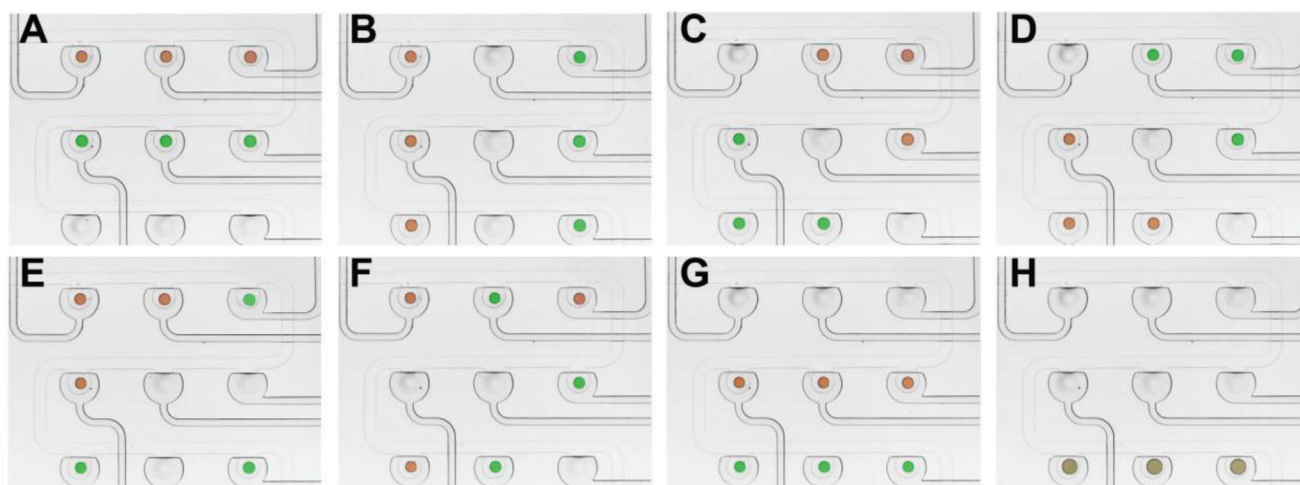


Figure 6. Automated droplet manipulation. A–G) A sequence of capture and ejection steps with bidirectional flow is used to form user-defined patterns, with droplets containing either red or green dye moved between different traps as shown in each panel. H) Pairwise merging of the droplet sets is finally performed to yield three identical mixed volumes. All images are extracted from Movie S9 (Supporting Information).

velocity is the vision system frame rate, which defines the spatial resolution for the droplet position measurements used for MDT actuation timing. At the given flow rate and video capture speed of 50 frames per second, average fluid displacement in the main channel between successive video frames is $\approx 10 \mu\text{m}$, introducing potentially significant errors in droplet position measurements.

2.10. Programmable Serial Dilution

Robotic liquid handlers are routinely used to perform microliter-scale serial dilutions for applications in chemistry and biology,^[17] enabling the programmable generation of discrete spatial concentration gradients of reagents and analytes for assays in immunology, microbiology, pharmacology, and beyond. Here we evaluate the capabilities of the MDT array technology to perform programmable serial dilution, opening the door to a wide range of nanoliter-scale on-chip assays where serial dilution is a required step. Specifically, the use of serial dilution for pH ladder generation is demonstrated. Control over sample pH is necessary for applications including droplet-based cell culture, biomolecular separations, and protein crystallization screening. While pH in conventional droplet microfluidic platforms is typically defined by the buffer conditions used during droplet generation, the ability to actively and controllably adjust solution pH within individual droplets can provide greater experimental flexibility and enable new operations that cannot be performed with static droplet platforms. Integrated mixers^[98,99] have been employed to actively adjust the pH in single-phase microfluidic systems, while techniques including on-chip electrochemical control^[100] and inter-droplet ion transport^[101] have been demonstrated for dynamic pH manipulation in droplet microfluidic platforms. Automated droplet manipulation using the MDT arrays offers a new approach to this challenge. The combination of software-defined droplet generation, capture, splitting, and merging sequences with flexible control over sample flow direction within the array enables arbitrary mixing steps to be performed as part of a serial

dilution process, thereby allowing initial droplets with high and low pH values to be metered and recombined to yield intermediate pH levels. To demonstrate this concept, cascade dilution was performed by combining droplet generation with repeated capture, splitting, and merging steps to form a seven-step pH ladder with a gradient ranging from pH 6 to pH 10.2. Starting with two sample solutions defining the desired pH range, individual nanoliter droplets with varying pH were generated through a sequence of up to six sequential droplet splitting steps and up to three merging steps, yielding final droplets containing different volume ratios of the initial solutions. Unused satellite droplets formed during the splitting steps were ejected through the inlet to minimize the potential for unwanted crosstalk between captured droplets. A logarithmic pH ladder was produced using this approach, as reflected in the resulting indicator dye intensities (Figure 7; Movie S10, Supporting Information).

Beyond the specific droplet operations explored here, the MDT traps can support other automated functionalities. For example, metering of different sample volumes from a given trap may be achieved by applying variable pressure inputs to the pneumatic control line. It is also possible to eject a trapped droplet when a mobile droplet or sample plug traverses the trap opening, allowing the MDT to serve as a nanoinjector to merge the two volumes within the main channel (Movie S11, Supporting Information). While this latter process often results in an initial droplet splitting event due to the co-ejection of both water and oil phases from the trap, the resulting oil film separating the resulting discrete volumes typically drains within several seconds to yield a single mixed droplet. Droplets may also be delivered to an exit port for off-chip collection, allowing fluid volumes processed within the array to be extracted for characterization using conventional benchtop instrumentation. For example, flow cytometry techniques such as fluorescence-activated cell sorting may be employed on droplets ejected from an MDT array by first processing the collected droplets in a water/oil/water double emulsion generator prior to introduction into the cytometer.^[102] Alternately, various approaches for analyzing collected droplets by mass

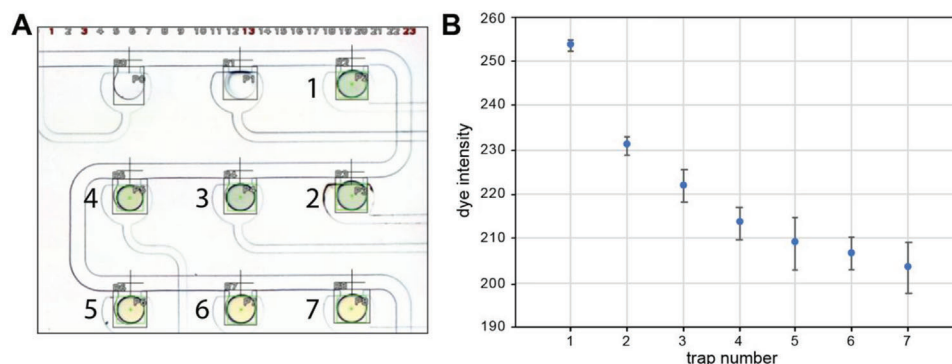


Figure 7. Programmable serial dilution for pH ladder generation. A) Image of processed droplets with pH-sensitive dye following pH gradient generation. Sample dilution is achieved by programmed droplet injection, splitting, and merging operations to yield a logarithmic seven-step pH ladder ranging from pH 6 to pH 10.2. B) Plot of dye intensity values from each trap given by the average value of the red and green image channels.

spectrometry have been reported.^[103–106] The automated system may also be used for applications in droplet-based cell culture. Compared with water-in-oil emulsions employing mineral oil with non-ionic surfactant, long-term cell culture within droplets demands the use of a gas-permeable fluorinated oil and biocompatible surfactant system to maintain cell viability. We have confirmed that droplets in fluorinated oil (FC-40) can be successfully manipulated in an MDT array (Movies S12 and S13, Supporting Information), with the generation, capture, and ejection of both small (0.3 nL) and large (1.0 nL) droplets achieved using an unmodified MDT chip. Because cell culture has been previously reported within picoliter-scale droplets using a similar fluorinated oil and surfactant system, we anticipate that cell viability may be readily maintained within the larger nanoliter-scale droplets explored in the present work.^[107]

While the devices explored here are limited to nine individual trap elements, arrays containing significantly higher well counts would expand the utility of the technology for high throughput applications. Array scaling is currently limited by two constraints. First, routing of the pneumatic lines used for membrane actuation imposes a minimum spacing between array elements. Current design rules employ 100 μm wide control lines with 200 μm spacing between adjacent lines to ensure reliable fabrication results. Given this constraint, the maximum number of traps that can reside within a 1 cm^2 array area is limited to ≈ 30 . While a modest increase in array density is possible by reducing the required control channel dimensions, a more scalable approach involves the addition of a dedicated PDMS routing layer to the chip design using multilayer PDMS soft lithography.^[108–110] This approach can serve to increase the space available for pneumatic channel routing, reduce the minimum spacing between traps, and allow control channels on different layers to cross one another as well as the traps themselves, enabling array densities on the order of 256 traps/ cm^2 in future designs. A second constraint in the current process involves fabrication challenges associated with the number of pneumatic inputs required for MDT actuation, since fabrication complexity scales the number of world-to-chip needle ports required for independent pressure control of each MDT element. In addition, the chip real estate required for control port integration and pneumatic control line routing can quickly dominate the total device area as array size increases. To address this limitation, various pneumatic microfluidic

multiplexers have been reported to provide control over large numbers of on-chip elastomer actuators with a minimal number of control inputs,^[111–113] offering a potential solution to the scaling challenge. For example, we have previously reported the design and implementation of an on-chip multiplexer topology capable of MDT actuation based on the pioneering work of Thorsen et al.^[113] that employs n inputs to control $2^{n/2}$ traps,^[69] offering a promising path toward future arrays with significantly higher trap densities through microfluidic large-scale integration.

3. Conclusion

The vision system-enabled MDT array platform represents a new microfluidic framework for automated droplet manipulation, enabling software-defined control over arbitrary sequences of complex droplet operations at the nanoliter scale. By directly modulating trap volume, the membrane displacement traps enable a range of droplet operations in a manner analogous to robotic stations employing automated pipetting for fluid control at the milliliter scale. The platform's simple chip design allows for the integration of individually-addressable traps and multiple sample inlets supporting dynamic on-demand droplet generation, as well as bidirectional flow control using an H-bridge valve topology for arbitrary droplet positioning within the system. The vision system used for automated droplet tracking was found to enable effective closed-loop control of the on-chip valves for fully automated operation of the system. Additionally, with combined with optimized timing control over the on-chip actuation elements, the Python-based library developed for user-defined scripting of complex droplet sequences served to provide precise control over each process step without user intervention, allowing hundreds of sequential operations to be performed with reliable outcomes. Overall, the platform offers flexible and deterministic control over the location and state of each individual nanoliter-scale droplet, enabling complex sequences of metering, sorting, and mixing operations to be performed with ease. The technology holds promise for a wide range of applications in biology, chemistry, materials science, and beyond by opening new opportunities for automated control over multi-stage chemical and biological assays performed within discrete nanoliter-scale droplets.

4. Experimental Section

Materials and Reagents: Microfluidic chips were fabricated by soft lithography using polydimethylsiloxane (PDMS) elastomer (Sylgard 184, Dow Corning). To characterize trap performance, the aqueous phase consisted of red and green food dye (McCormick) dissolved 1:20 in deionized water. The continuous phase consisted of light mineral oil with the addition of 0.01% w/w Span 80 surfactant for all experiments, except where noted. For experiments evaluating the performance of droplet manipulation in fluorinated oil, FC-40 (3M) with 0.01% Fluosurf surfactant (Darwin Microfluidics) was used as the continuous phase. All fluidic chip connections were made using Tygon microbore tubing (0.51 mm ID, 1.52 mm OD, Cole-Parmer) connected to 24 gauge needle segments inserted in access holes within each microfluidic device.

Chip Fabrication: The MDT array chips were fabricated by PDMS soft lithography. The device design consisted of an upper fluidic layer with fluid channels and membrane displacement traps and a lower control layer containing hydraulic channels for trap and valve actuation attached to a glass slide to seal the control layer microchannels and served as a rigid carrier for the assembly. Soft lithography molds for both the fluidic layer and control layer were prepared using silicon wafers patterned with SU-8 2015 photoresist (Kayaku). For both molds, the SU-8 was spin-coated at 500 rpm for 5 s, followed by 1150 rpm for 32 s, to yield a final feature height of 40 μm . To form the fluidic layer, PDMS (10:1 w/w base: curing agent) was poured over the corresponding silicon mold to a thickness of 5 mm, then cured in an oven at 80 $^{\circ}\text{C}$ for 25 min. Channel features in the fluidic layer were 100 μm wide, with circular trap features 200 μm in diameter yielding a trap volume of 1.3 nL. The PDMS was then peeled off the wafer, and cut into individual device sections with a scalpel. To form the control layer, a thin film of PDMS (20:1 w/w base: curing agent) was formed on the mold wafer by spin-coating at 500 rpm for 10 s, followed by 1500 rpm for 60 s, yielding a final thickness of 65 μm . Channel features in the resulting control layer were 100 μm wide and capped with a 24.8 μm thick membrane. The wafer was then baked at 80 $^{\circ}\text{C}$ for 5–7 min to partially cure the PDMS until tacky. Before separating the control layer from its mold wafer, the fluidic layer sections were aligned and bonded to the partially cured hydraulic layer. After assembling all of the multilayer structures, the wafer was cured at 80 $^{\circ}\text{C}$ for 24 h before peeling the PDMS from the wafer. Fluidic inlet and outlet ports, together with hydraulic access ports, were formed with a 0.5 mm diameter punch (Ted Pella). The control layer was sectioned with a scalpel to define the final chip dimensions, and the resulting chips were exposed to oxygen plasma for 30 s together with glass slides before pressing the surfaces together to facilitate a permanent bond. Finally, the devices were incubated at 80 $^{\circ}\text{C}$ for 24 h to restore native hydrophobicity to the PDMS surfaces following plasma treatment.

Vision System: A See3Cam CU30 USB camera (e-con Systems) was mounted to the trinocular receptacle of an SMZ745T microscope (Nikon). A generic X-Y stage allowed positioning of the microfluidic device in the camera's field of view. A generic LED light source and diffuser illuminated the device from below. The vision system was implemented in Python 3 using the OpenCV computer vision library running on a desktop PC. Droplet tracking was achieved using color thresholding functions within OpenCV to resolve individual blobs. User-defined experiments were coded in Python and controlled using the provided graphical interface. The interface allowed individual control of traps, pressure valves, and thresholding settings. It also showed the live video feed, detected blobs, user-defined trigger elements, and diagnostic information. Details of the vision system software and user interface are provided in Note S1 (Supporting Information).

Pressure and Flow Control: Elastomer membrane valves in the control layer were actuated using pressure inputs delivered through a pair of manifolds each containing a set of twelve two-way solenoid-controlled pneumatic valves (Clippard). The manifold input pressure was controlled through a pair of gas regulators (9892K12, McMaster-Carr), each supplied with a high-pressure air line, and a three-way solenoid valve to allow switching between the regulator outputs. The manifold board was pneumatically interfaced with the microfluidic chip through urethane tubing (0.06 in ID, 0.13 in OD, Clippard) and 24 gauge needle segments (Hamil-

ton) inserted into on-chip access holes. Control over the continuous-phase oil flow rate was achieved using an electronic pressure regulator (EPR-150, 0–150 psi range, Equilibar) to pressurize the head space of an off-chip oil reservoir. Pneumatic actuation of the MDT membranes was performed using a nominal pressure of 20 psi. When using multi-step actuation to avoid unwanted droplet splitting when ejecting larger droplets from a trap, the pressure was first set to 3.5 psi, and increased to 20 psi under software control when the droplet was observed to begin exiting the trap.

pH Gradient Generation: Sample solutions with different pH values were produced by electrolysis using a syringe pump (NE-8000, New Era Pump Systems, Farmingdale, NY) to withdraw tap water from a reaction vessel at a rate of 200 $\mu\text{L min}^{-1}$ through a steel needle, to which 24 V was applied by a DC power supply. A second electrode, formed from bare wire and submerged alongside the needle, was connected to the ground terminal of the supply. Starting with a 20 mL sample volume, 10 mL was withdrawn during electrolysis to serve as an acid solution, leaving 10 mL in the reaction vessel to serve as a base solution. A pH indicator dye (UI-100, Micro Essential Labs, Brooklyn, NY) was added at 15 % v/v to both solutions. The final pH values for the stock solutions were measured as pH 6.0 and 10.2, corresponding to solution colors of yellow and blue-green, respectively.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

automation, digital assays, droplets, microfluidics

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